The Antidiabetic Efficacy of Combined Extracts from Two Continental Plants: *Azadirachta indica* (A. Juss) (Neem) and *Vernonia amygdalina* (Del.) (African Bitter Leaf)

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Abstract: Polyherbal therapy is said to be a current pharmacological principle having the advantage of producing maximum therapeutic efficacy with minimum side effects. We assessed the antidiabetic efficacy and hence the impact on biochemical indices of toxicity by a combination of extracts from neem and bitterleaf. Thirty rats, 25 diabetic and 5 non-diabetic rats, were used for the study. The diabetic rats were divided equally into five groups and respectively treated: saline (diabetic control), extracts from neem and bitterleaf combined, neem only, bitterleaf only and chlorpropamide for a 24 day period. After oral administration of the first dose of extract (400 mg kg^{-1} b.w.) and chlorpropamide (4.286 mg kg^{-1} b.w.), blood glucose was monitored *in vivo* at various time intervals for 9 h, thereafter daily administration continued for 24 days. Whereas single dose treatment with neem only showed peak reduction (28.56%) an hour after, treatments with combined extracts, bitterleaf and chlorpropamide had their peak reductions all at the 7th hour (24.78, 47.31 and 60.51%, respectively). Percentage reductions in blood glucose relative to their initial values at the end of treatment were 71.05, 44.95, 88.63 and 75.83 for combined extract, neem, bitterleaf and chlorpropamide respectively. The decrease in blood glucose for the groups treated with combined extracts and bitterleaf only compared well (p<0.01) with chlorpropamide and non diabetic control, but not with neem alone. Determination of markers of hepatotoxicity in serum including GPT and GOT activities, total protein, albumin and urea indicated that, of the four treatments, neem provides the best protection against hepatic dysfunction. In the group treated with combined extracts these alternate selective advantages of neem and bitterleaf were expressed as a positive synergy, hence more beneficial than individual treatments.

Key words: Polyherbal therapy, *Azadirachta indica*, *Vernonia amygdalina*, diabetes mellitus, blood lucose

INTRODUCTION

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in international trade[1]. In certain African countries for instance, up to 90% of the population still relies exclusively on plants as a source of medicines[2]. As a consequence, the World Health Organization (WHO) had in one of its charters in Geneva recommended further investigation into this area, particularly as it concerns chronic and debilitating diseases such as diabetes mellitus[3].

A complex disease like diabetes mellitus, where little is talked about in aspects of prevention and curation, but rather management, there is an increased focus on plants in the search for appropriate hypoglycemic/antihyperglycemic agents. Firstly, because of leads provided by traditional medicine to natural products that may be better treatments than currently used conventional drugs[4]. Secondly the plants by secondary metabolic means contain a variety of herbal and non-herbal ingredients that are thought to act on a variety of targets by various modes and mechanisms[5]-given the multi-factorial pathogenicity of the disorders. Moreover, polyherbal therapies the combination of various types of agents from different plant sources, can be used to enhance efficacy. According to Tiwari and Rao[5] polyherbal therapies have the synergistic, potentiative, agonistic/antagonistic pharmacological agents within themselves, that work together in a dynamic way to produce therapeutic efficacy with minimum side effects.

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It is in this light that this work was designed to investigate the antidiabetic efficacy of combined extracts from two most widely used plants *Azadirachta indica* and *Vernonia amygdalina*, employed traditionally in the management of diabetes in Asia and the African sub-Region, respectively.

A. indica, normally found in the Indian subcontinent and the dry forest areas of South and Southeast Asia including Pakistan, Sri Lanka, Thailand, Malaysia and Indonesia, but cultivated in most other countries of the world belongs to the plant family Meliaceae[6]. Biswas *et al.*,[7] have reviewed the biological activities and medicinal properties of the plant and articulated, the hypoglycemic effects of its leaves, stem and bark and seed oil.

On the otherhand, *V. amygdalina* is a compositae both of wild and domestic browse in Africa. The plant has a reputation in its use in traditional management of diabetes in Southern Nigeria[8]. An earlier study in our laboratory compared the effect of extracts from the two plants separately on beta cell integrity in rats pretreated with alloxan, a beta cell destructive agent[9]. The present study compared the antidiabetic efficacies of combined extracts from the two plants with the plant extracts taken singly and also with a conventional sulfonylurea, chlorpropamide. Since hyperglycemia increases the generation of free radicals by glucose auto-oxidation and this increment of free radicals usually would lead to organ damage[10], we also assessed indices of liver function/toxicity at the end of these treatments.

MATERIALS AND METHODS

Preparation of plants extracts: Matured leaves of *V. amygdalina* and *A. indica* were obtained from the Endocrine Research Farm of the University of Calabar, Calabar and University of Calabar staff quarters, respectively. Four hundred grammes (400 g) of each was separately mixed with 80% ethanol and homogenized using an electric homogenise. The homogenates were allowed in a refrigerator (4°C) for 48 h and thereafter filtered using a chess material. The filtrates were concentrated at low temperature (37°C) under reduced pressure using a rotary evaporator to one tenth of their original volumes. These were then allowed in a water bath at 37°C for complete evaporation to dryness[11] yielding 36.48 g (9.12%) and 24.84 g (6.21%), respectively for *V. amygdalina* and *A. indica*.

Experimental animals and alloxan-induction of diabetes: Thirty healthy albino Wistar rats (males and females) obtained from the animal house, Department of Zoology, University of Calabar, Calabar after due permission from the Faculty Animal Ethics Committee were used for the study. The rats were allowed one week to acclimatize to Biochemistry departmental animal house, where they were housed throughout the experiment in polycarbonated cages. The animal housing facility was maintained at standard conditions: temperature (28±2°C), relative humidity (50±5%) and a 12 h light/dark cycle. Water and commercial palletized diet were available to the animals *ad libitum* throughout the treatment period. Twenty-five of the animals were induced with diabetes by injecting 150 mg kg^{-1} body weight of alloxan monohydrate (Sigma St. Louis, MO, USA) intraperitoneally. Ninety-six (96hrs) after, rats confirmed diabetic (i.e., random blood glucose ≥200 mg dL^{-1}) were selected for the experiment.

Extract and drug administration: Before use, the extracts were reconstituted in normal saline (vehicle) and administered orally via gastric intubation at a dose of 400 mg kg^{-1} b. w. for single extract treatment and 200 mg kg^{-1} each in combined extracts treatment. Chlorpropamide was administered at a dose of 14.286 mg kg^{-1} b. w. to simulate human regimens. The dosage of the extracts was determined from preliminary studies in our laboratory. The controls received normal saline (placebo).

Experimental design: The design consisted of 30 rats (25 surviving diabetic rats and 5 normal rats) divided into 6 groups of 5 rats each. Groups 1 and 2, normal and diabetic control rats given placebo treatment, Group 3: diabetic rats treated with combined extracts from *A. indica* and *V. amygdalina*, Group 4: diabetic rats treated with extract from *A. indica* only, Group 5: diabetic rats treated with extract from *V. amygdalina* only and Group 6: diabetic rats administered chlorpropamide. After first oral dose administration of extracts/drug, *in vivo* measurement of blood glucose was done with blood obtained from tail vein of the rat at 1, 3, 5, 7 and 9 h using One Touch ® Glucometer (Lifescan, Inc., 1995 Milpitas, California 95035, USA). The oral administration then was continued twice per day in a 12 h cycle (6: am and 6: pm) for 24 days. At the end of the 24 day period, the animals were fasted for 12 h, then anaesthetized under chloroform vapour and dissected. Whole blood obtained by cardiac puncture into plane tubes was allowed to clot for about 2 h and thereafter centrifuged (3,000 g for 10 min) to remove cells and recover serum, which was used for the biochemical assays.

Biochemical analysis: GPT (Glutamic Pyruvic Transaminase) and GOT (Glutamic Oxaloacetic Transaminase) assay kits were obtained from Randox Laboratories Ltd., Admore Diamond Road, Crumin Co. Antrim, UK. The assay methods are based on transamination reaction in which the concentrations of pyruvate (GPT) and oxaloacetate (GOT) hydrazones formed in the presence of 2,4 dinitrophenyl-hydrazone are measured at 546 nm[12]. Assay kits for total protein, albumin and urea determination were obtained from DIALAB Produktion und Vertrieb von chemisch-technischen, produktion und laborinstriementen Gesellschaft m.b.H. A-1160 Wien-panikengasse, Austria. Total protein in samples were measured by the method based on biuret reaction[13] whereas that of albumin relies on the binding of bromcresol green (BCG) to albumin[14]. Urea determination in the method was based on urea hydrolysis by urease enzyme[14].

Statistical analysis: The results were analysed for statistical significance by one-way ANOVA using the SPSS statistical program and Post Hoc Test (LSD) between groups using MS excel program. All data were expressed as Mean±SEM. P values <0.01 and 0.05 were considered significant.

RESULTS

Effect of extracts/drug on blood glucose level: Table 1 shows effect of a single dose treatment on blood glucose determined at various time intervals for 9 h. The values in parenthesis are percent change in blood glucose (%BG):

$$\%BG = G_t \times 100/G_o$$

Where G_o and G_t are glucose levels at time o and t, respectively. Four days after alloxan-treatment, blood glucose of diabetic rats was significantly raised by 5.4-9 times the value of the normal control rats. First dose treatment caused reduction over the intervals monitored but with significant peak reductions at 1hour for *A. indica* treated group (28.56%) and at 7th hour for combined extracts treatment (24.78%), *V. amygdalina* alone (47.31%) and chlorpropamide (60.51%) treated groups (Fig. 1). At the end of the chronic treatment period (24 days) decreases in blood glucose level relative to their initial values (96 h past alloxan values) of 71.05, 44.95, 88.63 and 75.83% were observed in groups treated with *A. indica* and *V. amygdalina* combined, *A. indica*, *V. amygdalina* and chlorpropamide, respectively (Fig. 2). These reductions were significant at p<0.01 except in the group treated
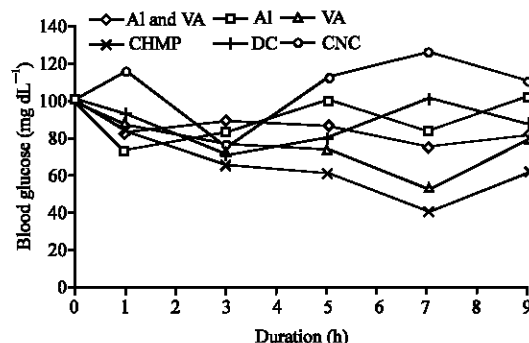

Fig. 1: Percentage of effect of plant extracts/drug on blood glucose levels monitored at various time intervals for 9 h, compared with 0 h data in diabetic rats (%). Values are mean percent of blood glucose concentrations (n = 5). AI-A.indica, VA = V. amygdalina, CHMP = Chlorpropamide, DC = Diabetic control and NC = normal control
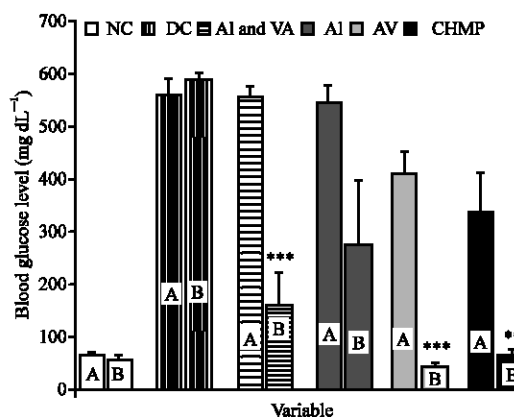

Fig. 2: Blood glucose level after 24 day t/m with combined extracts, *A. indica* only and *V. amygdalina* only and chloropropamide in diabetic rat models. A, B: Blood glucose level before and after 24 day treatment. **p<0.01; ***p<0.001 vs A

with *A. indica*. The 24 day blood glucose levels show significant reduction (p<0.01) in all the treated groups compared to the diabetic control. Compared to normal control, *A. indica* treated group yet had blood glucose significantly higher (p<0.05), unlike the other 3 treatments that compared well with it at (p<0.05) (Fig. 3).

Effect of drug/extract treatment on some serum biochemical indices: Table 2 shows results of

Table 1: Effect of drug/plant extracts on the blood glucose level (mg kg^{-1}) of alloxan induced diabetic rats

GRP	0 h	1 h	3 h	5 h	7 h	9 h
GRP 1	62.0±5.29	69.8±4.13	56.8±4.01	66.8±4.58	75.4±5.55	66.2±4.77
	(100)	(114.84±8.17)	(74.59±15.47)	(112.16±14.09)	(125.09±13.40)	(109.37±10.13)
GRP 2	558.5±29.95	512.8±34.34	396.5±61.96	445.0±56.36	556.0±41.38	484.3±95.91
	(100)	(92.07±4.11)	(71.41±9.56)	(79.96±8.23	(100.54±8.78)	(87.30±15.63)
GRP 3	558.6±19.70	459.6±29.70	495.6±46.91	482.6±60.53	418.2±74.51	451.2±66.47
	(100)	(82.33±5.40)	(88.98±8.48	(86.42±10.84)	(75.22±13.81)	(80.96±12.39)
GRP 4	545.4±34.11	375.8±63.83	450.0±40.87	531.2±31.30	435.4±29.99	544.2±20.73
	(100)	(71.44±14.54)	(83.05±6.56)	(100.00±11.71)	(82.59±11.82)	(80.96±12.39)
GRP 5	412.2±40.60	353.4±76.64	315.0±56.96	308.6±54.76	213.0±31.12	314.4±58.67
	(100)	(87.13±19.20)	(77.00±12.98)	(73.78±8.08)	(52.69±8.38)	(79.60±19.15)
GRP 6	335.2±74.94	302.2±92.00	242.6±82.72	233.2±96.20	142.8±52.38	236.0±98.22
	(100)	(82.68±12.83)	(64.73±12.19)	(61.26±11.71)	(39.49±6.67)	(61.87±14.58)

Mean±SEM, n = 5

Table 2: Effect of drug/plant extracts on some serum biochemical indices

GROUP	GPT (Unit L^{-1})	GOT (Unit L^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Urea (mg dL^{-1})
GRP 1	4.22±0.67	5.85±1.33	7.68±0.15	2.88±0.19	36.85±4.11
GRP 2	15.50±0.73	74.00±5.14	7.17±0.24	2.84±0.18	118.98±10.38
GRP 3	6.40±0.70*	13.88±2.88*	7.15±0.36	3.17±0.11	73.88±35.44
GRP 4	6.88±0.66*	8.34±1.75*	7.54±0.37	3.35±0.18*	60.08±8.47**
GRP 5	4.98±0.97*	21.50±9.98*	7.77±0.24	3.25±0.13	66.06±16.43**
GRP 6	4.40±0.25*	24.10±8.37*	7.77±0.25	3.05±0.23	69.35±40.00

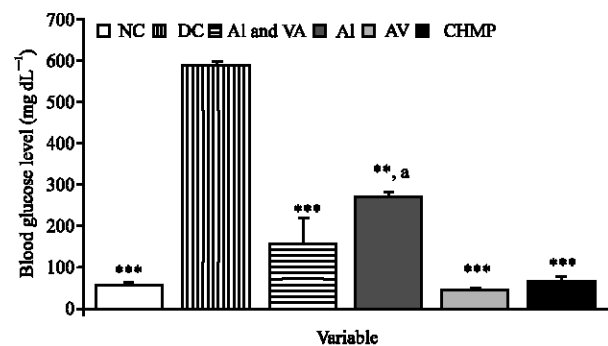Mean±SEM, n = 5, *p<0.05 vs DC **p<0.05 vs NC

Fig. 3: Blood glucose level after 24-day treatment with extracts, *A. indica* and *V. amygdalina combined*, *A. indica*, *V. amygdalina* and chloropropamide in diabetic rat models. **p<0.01; ***p<0.001, vs NC, a = p<0.05 vs DC

parameters assayed in serum of the sacrificed animals after 24 day treatment including GPT and GOT activities, protein total, albumin and urea levels. Serum GPT and GOT activities which were significantly raised (p<0.05) by 3.6 and 12.7 times, respectively relative to the normal control, became significantly reduced upon treatment. GPT activity significantly reduced (p<0.05) to 2.4, 2.2, 3.1 and 3.5 times the diabetic control value in rats treated with combined extracts, *A. indica*, *V. amygdalina* and chlorpropamide, respectively. In the same order GOT activity, respectively reduced to 5.3, 8.9, 3.4 and 3.1 times the

value of the diabetic control. However, when compared with normal control and standard drug, extract treatments both singly and combined showed non significant differences in the aminotransferase activities. Serum urea which was 3.2 times high in diabetic control group compared to normal control, was significantly reduced (p<0.05) with the four different treatments. Compared to the normal control, urea level in groups treated with combined extracts and chlorpropamide were non-significant (p<0.05), whereas levels in groups treated with *A. indica* and *V. amygdalina* were significantly higher. Aside treatment with *A. indica* which significantly raised (p<0.05) albumin levels compared to diabetic control, serum protein total and albumin levels observed no changes.

DISCUSSION

The antihyperglycemic effect of ethanolic extracts of *A. indica* and *V. amygdalina* combined, *A. indica*, *V. amygdalina* and chlorpropamide have been evaluted in this study. The results are consistent with Sonia and Srinivasan [17] for *A. indica*, Atangwho *et al* [11] for *V. amygdalina* and Davis and Granner[17] for chlorpropamide. However, from the present investigation the effect of *A. indica* on blood glucose was less than that of chlorpropamide, but significantly higher than normal control; *V.amygdalina* effect was more than chlorpropamide and even the normal control, though not significantly. Whereas effect of combined

extracts on blood glucose compares fairly well with that of chlorpropamide, which itself compares well with normal control. Within the treatment period, combined extracts effect on blood glucose appears a positive synergy: *A. indica* could not reverse the hyperglycemic state to normoglycemic status, while on the other hand *V. amygdalina* tended towards hypoglycemia. In combined form, the extracts tend to compliment each other thereby producing the desired normoglycemia.

This observation may buttress the proposition of Tiwari and Rao[5] as per advantage of polyherbal therapies over monotherapy. Although not fully understood, several reports have attempted insights into the hypoglycemic mechanisms of these plants. There is a report that *A. indica* does not modulate insulin effect on glycogen metabolism[20] rather it blocks the inhibitory effect of serotonin on insulin secretion/release in pancreas of rats mediated by glucose, eventually glucose control. Sonia and Srinivasan[17] in their report presupposed increased peripheral glucose uptake by an inhibition on the action of insulin by inhibiting glycogenesis. We have suggested in our earlier report on *V. amygdalina* that two possible mechanisms exist: one targeting insulin production from the islet cells and the second on peripheral carbohydrate mechanism[11]. Mechanisms involving insulin production are usually more potent. It's not surprising therefore that *V. amygdalina* tends to be the most potent hypoglycemic agent. More so, that other plants of the same family-compositae, such as *Chrysanthemum coronariun* has shown potent hypoglycaemic and anti-hyperglycaemic activity[10].

Serum markers of hepatotoxicity-GOT and GPT were also determined. Diabetic control rats showed significantly elevated enzyme activities in serum compared to normal control. The observation is collaborative with kim *et al.*[10]. Upon treatment with the extracts and drug the activities of both enzymes become reduced significantly. Again, this agrees with hepatoprotective effect of *V. amygdalina*[22] and *A. indica*[23,24] against drug induced injury reported in earlier studies, although in this study the hepatotoxicity was diabetes-induced. Interestingly, in this study, *A. indica* appears to have a better protection against hepatotoxicity as it produced highest % reduction in GOT activity. On combination, this potency seems to boost the effect of *V. amygdalina* by increasing the percentage protection-another positive synergistic effect.

Serum total protein, albumin and urea, markers of liver synthetic ability were assessed after treatment for 24 days. Urea levels raised significantly in diabetic control group compared to normal control agrees with our earlier report[11]. All treatments reduced the hitherto raised levels of urea, but only the groups treated with combined extracts compared well with normal control and chlorpropamide. Combined extract may modulate protein breakdown in diabetes mellitus more effectively. Significant increase in albumin levels in *A. indica* treated groups confirms its purported most potent hepatoprotective effect, given that albumin is exclusively synthesized in the liver. It's evident from the results of this work, that whereas *V. amygdalina* alone is most potent in blood glucose reduction mechanism and *A. indica* most potent in protecting the liver against damage in diabetic states. Only a combination of the two extracts provided the wholistic efficacy desired in management of diabetes. Further studies to confirm this relative advantage of polytherapy is suggested.

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